

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 89304371.1

(51) Int. Cl.⁴: **A 61 L 25/00**

(22) Date of filing: 28.04.89

(30) Priority: 02.05.88 US 189187

(43) Date of publication of application:
08.11.89 Bulletin 89/45

(84) Designated Contracting States:
AT BE CH DE ES FR GB GR IT LI LU NL SE

(71) Applicant: **PROJECT HEAR**
801 Welch Road
Palo Alto California 94304 (US)

MATRIX PHARMACEUTICAL INC.
1430 O'Brien Drive Suite H
Menlo Park California 94025 (US)

(72) Inventor: **Sierra, David H.**
1040 42nd Street South
Birmingham Alabama 35222 (US)

Luck, Edward E.
216 Robin Way
Menlo Park California 94025 (US)

Brown, Dennis M.
211 Robin Way
Menlo Park California 94025 (US)

(74) Representative: **Harrison, David Christopher et al**
MEWBURN ELLIS & CO 2/3 Cursitor Street
London EC4A 1BQ (GB)

(54) Surgical adhesive material.

(57) The present invention provides a surgical adhesive comprising, in an aqueous composition, patient autogenous plasma, collagen, thrombin, and optionally, an antifibrinolytic agent. The present adhesive is formed from the patient's plasma without the use of any added reagents for concentration or isolation of the fibrinogen. Conveniently, the adhesive is formulated as a two-part composition which is mixed together just prior to use.

EP 0 341 007 A2

08/479,038
A2
#36

Description

SURGICAL ADHESIVE MATERIAL

The field of the present invention relates to surgical adhesives.

Early surgical adhesive formulations based on fibrinogen suffered from a number of disadvantages. The fibrin solutions necessarily contained a high content of fibrinogen, about 8-10%, which could only be prepared from fibrinogen lyophilizates with difficulty. This so-called cryoprecipitate was relatively unstable and had to be stored at temperatures below -20°C until used. Formulations to improve the stability of the cryoprecipitate included adding inhibitors of plasminogen activator or albumin. The fibrinogen concentrates were mixed with thrombin just prior to application to the wound.

Other formulations of surgical adhesives had a pore structure based on collagen for covering wounds wherein a non-woven fabric consisting of collagen fibers was applied to the wound. The fabric was fixed to the wound using a fibrinogen-thrombin mixture which was applied to either the inner or outer side of the collagen fabric. However, the fibrinogen was found to coagulate rapidly and thus not to effectively penetrate into the collagen fabric.

The current formulations use patient autogenous fibrinogen glues together with thrombin. While the use of autogenous fibrinogen avoids problems with rejection of the material, the adhesives require relatively large quantities of patient blood. Further, the processing times range from an hour to overnight, and require both the equipment and expertise of a hospital clinical blood laboratory with trained technicians. Additionally, a number of reagents are introduced into the blood to fractionate and concentrate the fibrinogen and related proteins from the plasma in some patient autogenous fibrin glue (AFG) formulations.

Relevant Literature

U.S. Patent No. 4,650,678 describes a solid fibrinogen formulation which contains a substance having a urea or guanidine radical to increase the solubility and viscosity of fibrinogen solutions. U.S. Patent No. 4,600,574 describes a surgical adhesive based on a flat material consisting of collagen, gelatin or polysaccharide impregnated with a solution of fibrinogen and Factor XIII, which material is lyophilized to form a matrix.

SUMMARY OF THE INVENTION

The present invention provides a surgical adhesive comprising, in an aqueous composition, patient autogenous plasma, collagen, thrombin, and optionally, an antifibrinolytic agent. The present adhesive is formed from the patient's plasma without the use of any added reagents for concentration or isolation of the fibrinogen. Conveniently, the adhesive is formulated as a two-part composition which is mixed together just prior to use.

DESCRIPTION OF SPECIFIC EMBODIMENTS

A surgical adhesive using patient autogenous plasma in conjunction with collagen and thrombin is provided. The plasma can be used directly or as a plasma cryoprecipitate in which the fibrinogen has been concentrated without the use of any added reagents.

The surgical adhesive comprises in an aqueous composition patient autogenous plasma, collagen in an amount sufficient to thicken the composition, thrombin in an amount sufficient to catalyze polymerization of fibrinogen present in the plasma and, optionally, an antifibrinolytic agent in amount sufficient to retard degradation of the resulting adhesive clot. The surgical adhesive is conveniently formulated as a two-part composition in which plasma and collagen constitute the first component, and thrombin together with an anti-fibrinolytic agent, constitutes the second component.

Patient autogenous plasma provides a source of fibrinogen which provides the adhesive component of the composition. The plasma can be used "as is" after standard preparation which includes centrifuging out cellular components of blood. Alternatively, the plasma can be further processed to concentrate the fibrinogen to prepare a plasma cryoprecipitate. The plasma cryoprecipitate can be prepared by freezing the plasma for at least about an hour at about -20°C, and then storing the frozen plasma overnight at about 4°C to slowly thaw. The thawed plasma is centrifuged and the plasma cryoprecipitate is harvested by removing approximately four-fifths of the plasma to provide a cryoprecipitate comprising the remaining one-fifth of the plasma. Optionally, the plasma cryoprecipitate can be mixed with an equal volume of approximately 10% CaCl₂ solution. Approximately 0.5 ml to about 1.0 ml of either the plasma or the plasma-cryoprecipitate provides about 1 to 2 ml of adhesive composition which is sufficient for use in middle ear surgery.

Collagen, preferably hypoallergenic collagen, is present in the adhesive in an amount sufficient to thicken the adhesive composition. The collagen may be atelopeptide collagen or not. In addition to thickening the composition, the collagen augments the fibrin by acting as a macromolecular lattice work or scaffold to which the fibrin network adsorbs. This gives more strength and durability to the resulting glue clot with a relatively low concentration of fibrinogen in comparison to the various concentrated autogenous fibrinogen glue

formulations (i.e. AFGs). The amount of the collagen can be varied to provide adhesives of differing viscosities and strengths, depending on the particular application for the adhesive. Usually, the collagen is a flowable composition dispersed in phosphate buffered saline to provide a final concentration in the adhesive formulation of from about 5 mg/ml to about 30 mg/ml, more usually about 10 mg/ml to about 20 mg/ml, most usually about 15 mg/ml. Collagen is commercially available from a variety of sources, including Collagen Corporation (which sells hypoallergenic collagen under the tradename Zyderm). Collagen is generally available in a soft paste form, packaged in a sterile syringe. Further descriptions of collagen are found in U.S. Patent No. 4,233,360.

Thrombin acts as a catalyst for fibrinogen to provide fibrin, an insoluble polymer. Thrombin is present in the surgical adhesive in an amount sufficient to catalyze polymerization of fibrinogen present in the patient plasma. Usually the thrombin is present in the adhesive composition in a concentration of from about 1 to about 1000 NIH units (NIHu) of activity, usually about 100 to about 500 NIHu, most usually about 200 to about 300 NIHu. The thrombin can be from a variety of host animal sources, conveniently bovine. Thrombin is commercially available from a variety of sources including Parke Davis, usually lyophilized with buffer salts and stabilizers in vials which provide thrombin activity ranging from 1000 NIHu to 10,000

NIHu. The thrombin is usually prepared by reconstituting the powder by the addition of either sterile distilled water or isotonic saline.

Usually the surgical adhesive will additionally comprise an effective amount of an antifibrinolytic agent to enhance the integrity of the glue clot as the healing processes occur. A number of antifibrinolytic agents are well known and include aprotinin, Cl-esterase inhibitor and ϵ -aminocaproic acid (EACA). ϵ -aminocaproic acid, the only antifibrinolytic agent approved by the FDA, is effective at a concentration of from about 5 mg/ml to about 40 mg/ml of the final adhesive composition, more usually from about 20 to about 30 mg/ml. EACA is commercially available as a solution having a concentration of about 250 mg/ml. Conveniently, the commercial solution is diluted with distilled water to provide a solution of the desired concentration. That solution is desirably used to reconstitute lyophilized thrombin to the desired thrombin concentration.

For dental or orthopedic applications, inorganic minerals or mixture of inorganic minerals, naturally occurring or synthetic, desirably hydroxyapatite or minerals found in bone powder or chips may be added to the formulation, most conveniently to the plasma fraction of component 1. The mineral(s) are present in a volume ratio to the collagen component of from about 1:1 to about 4:1 depending upon the desired flow characteristics or intended use and site. Additionally, viable osteoblasts may be harvested from a donor site and incorporated into the composition, conveniently in component 1, for use in transplantation.

The surgical adhesive may additionally contain an antibiotic. The antibiotic may be incorporated into the collagen component if the antibiotic is a liquid. Alternatively, the antibiotic may be suspended in the plasma fraction of component 2 if it is in powder form. The therapeutic dose levels of a wide variety of antibiotics for use in drug release systems are well known. See for example, *Collagen*, Vol. III, Biotechnology; Marcel E. Nimni, Ph.D., Editor, CRC Press, Inc. (1988) pp. 209-221, and the references cited therein. Anti-microbial agents are particularly useful for compositions applied to exposed wound repair sites such as sites in the mouth or to compromised wound sites such as burns.

The surgical adhesive is conveniently formed by mixing two components just prior to use. The first component comprises the autogenous plasma together with collagen. That component is conveniently prepared by mixing the plasma with collagen to form a substantially uniform composition under low or no shear conditions at ambient or lower temperatures. Conveniently, using two syringes joined by a syringe-to-syringe connector having about a 1 mm or less diameter opening, substantial uniformity can be achieved with simple, generally available equipment. Generally, about 5 to 10 passes through the opening is sufficient. This component can be prepared during surgery or up to 8 hrs. prior to surgery, when stored at room temperature. Alternately, the plasma fraction may be collected and prepared up to one week prior to mixing with the collagen fraction. The second component comprises thrombin. If an antifibrinolytic agent is present in the composition, it is usually mixed with the thrombin as part of component 2. Component 2 can be stored for about 8 hrs. at room temperature, for about 2 days at about 4°C. or for up to a week when frozen at -20°C.

The two components are mixed just prior to the application to the patient. The components may be formulated with concentrations that allow mixing the components in substantially equal volumes to simplify the final preparation of the adhesive. Conveniently, a dual-syringe holder with a disposable mixing tip can be used. Alternatively, the two components can be mixed using two syringes as described above.

The surgical adhesive can be used in applications where prior art surgical adhesives were previously used. The material can be used as a soft tissue augmentor or soft tissue substitute in plastic reconstructive surgery. The adhesive may be also used to attach skin grafts to a recipient site without the use of sutures or with a reduced number of sutures, or as a growth matrix for transplanted intact osteoblasts in bone repair and reconstruction. The adhesive can also be used for applications such as ossicular chain reconstruction, nerve anastomosis or other situations where repair by sutures is impossible or undesirable, or as a wound dressing. The surgical adhesive may be applied in a number of ways determined by the surgical indication and technique.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Example 1

5

Preparation of Adhesive

To prepare a preferred formulation of the adhesive composition, the following protocol was followed. Patient's blood (5 cc) was collected in a citrated vacuum blood collection tube (Vacutainer) by venipuncture. The blood was centrifuged for 10 min at 4000 rpm. About 0.5 cc plasma was removed from the Vacutainer with a 1 cc syringe. A syringe-to-syringe connector (20-gauge) was used to mix 0.5 cc collagen (Zyderm I, Collagen Corporation) with the 0.5 cc patient's plasma for about 5 to 10 passes. Component 1 was then ready for use or was stored for up to about 6 to 8 hrs. at room temperature.

Component 2 was prepared by drawing 1 cc of a solution of 250 mg/ml of aminocaproic acid into a 12-cc syringe. Nine cc of Water for Injection, U.S.P., was drawn into the same syringe to provide a 25 mg/ml concentration of aminocaproic acid. Two cc of that solution was added to 1000 NIH units of bovine thrombin (Thrombostat, Parke-Davis) and drawn into a 1 cc syringe. Component 2 was ready for use or was stored, as described previously.

At that point, either the two syringes were mounted into a dual-syringe dispenser/mixer or modified spinal tap needles were attached to the individual syringes to mix and apply the components.

Example 2

25

Concentrations and Gelation Time Effects In Vitro

The following materials were used to determine gelation time in vitro:

- lyophilized fibrinogen from Sigma Chemical Company, bovine source, cat. # F4753, reconstituted to desired concentrations with Ringer's solution;
- collagen Zyderm Collagen Implant 1 without lidocaine by Collagen Corporation at 35 mg/ml, diluted as needed by Ringer's solution; and
- lyophilized thrombin Thrombostat by Parke-Davis, reconstituted with Sterile Water for Injection, U.S.P. to 100 NIH units per ml. (1 NIH unit (10 μ l) was delivered to each test formulation.)

Human plasma was collected using citrated Vacutainers, then centrifuged for 10 min at 4000 rpm. A 1 ml test solution of each formulation was maintained at 37°C using a circulating water bath in each assay.

Tables 1 and 2 illustrate the results. As used in the Tables, [Fibro] [collagen] indicate the concentration of clottable lyophilized fibrinogen and collagen, respectively, dissolved in Ringer's Solution and Gel time is the amount of time for gelation of the clot. The collagen and fibrinogen concentrations listed are the final concentrations in the test adhesive mixture.

40

TABLE 1
LYOPHOLIZED FIBRINOGEN WITHOUT
COLLAGEN

45

	[Fibro] (mg/ml)	Gel Time (sec)	Comments
50	5	19	solid clot
	2.5	22	solid clot
	1.25	30	solid clot
	0.6	40	solid clot
	0.3	50	solid clot
55	0.15	90	fluid clot, transparent
	0.07	210	fluid clot, fragile
60	0.035	270	fluid clot, fragile
	0.017	--	no visible clot formation

65

TABLE 2
LYOPHOLIZED FIBRINOGEN WITH COLLAGEN
AT 6 mg/ml

[Fibro] (mg/ml)	Gel Time (sec)	Comments
2.5	7	solid clot
1.25	8	solid clot
0.6	9	solid clot
0.3	14	solid clot
0.15	20	solid clot
0.07	--	no apparent clotting effect
0.035	--	no apparent clotting effect

As demonstrated using fibrinogen without collagen (Table 1), there was some clotting effect at 0.07 and 0.035 mg/ml fibrinogen concentration. However, due to the fragility and fluidity of the resultant clots and comparison to the viscosity of a collagen-alone control using 6 mg/ml collagen, it was difficult to discern any clotting effect at these lower fibrinogen concentrations. As shown in Table 2, solid clots were achieved at fibrinogen concentrations of from 2.5 to 0.15 mg/ml using 6 mg/ml collagen.

TABLE 3
LYOPHOLIZED FIBRINOGEN WITH COLLAGEN
AT 15 mg/ml

[Fibro] (mg/ml)	Gel Time (sec)	Comments
2.5	--	instantaneous gelation
1.25	10	solid clot
0.6	15	solid clot
0.3	25	solid clot
0.15	30	solid clot
0.07	40	fluid and fragile clot
0.035	45	fluid and fragile clot
0.017	120	fluid and fragile clot
0.008	--	no apparent gelation

This data demonstrated the practical limits for concentrations that can be used under these experimental conditions for reconstituted lyophilized bovine fibrinogen. In particular, compositions with concentrations of lyophilized fibrinogen as high as 2.5 mg/ml gelled instantly and were not useful. Concentrations of 0.07 mg/ml or less produced a fluid and fragile clot. Formulations using between 1.25 and 0.15 mg/ml fibrinogen together with 15 mg/ml collagen produced useful adhesive compositions. Higher concentrations than 15 mg/ml of collagen may be used successfully. However, it is difficult to objectively assess gelation time due to the limitations of current test equipment in evaluating test solutions of relatively high viscosity.

A study was performed to determine the dilutions of plasma that were sufficient for proper clot formation. The results are shown in Table 4. Other than the plasma dilution, the conditions were the same as those described for the studies illustrated in Tables 1-3.

TABLE 4

PLASMA WITH RINGER'S AS DILUENT

	Plasma ml	Ringer's ml	Gel Time sec	Comments
5	1.0	0	15	solid clot
	0.75	0.25	12	solid clot
10	0.5	0.5	20	solid clot
	0.33	0.67	30	fluid clot
	0.25	0.75	35	fluid clot
	0.12	0.87	—	no gelation, small clots
15				

The purpose of this study was to compare the effect of various plasma dilutions. Since one is, in essence, diluting the plasma with collagen to form the adhesive, the effect of collagen in comparison to buffer solution as diluents on gelation time and clot quality was studied. Plasma was mixed with ZCI collagen (35 mg/ml) to determine useful ranges of plasma fibrinogen concentrations.

TABLE 5

PLASMA WITH ZCI COLLAGEN AT A CONCENTRATION OF 35 mg/ml

	Plasma (ml)	Collagen (ml)	[Collagen] (mg/ml)	Gel Time (sec)	Comments
	0.75	0.25	8.75	15	*
30	0.5	0.5	17.5	10	solid clot
	0.33	0.67	23.1	30	solid clot
	0.25	0.75	26.2	100	small clots

*Collagen congealed into a small clot within the gel, which was difficult to evaluate.

As shown in Table 5, combining volumes of plasma with 35 mg/ml collagen in ratios between about 1:1 to about 1:2 plasma:collagen produced useful adhesives. This study demonstrated that the presence of collagen as a plasma "diluent" actually allows plasma fibrinogen to form a solid clot at lower fibrinogen concentrations than plasma fibrinogen at the same concentration but without collagen.

The effect of the calcium ion concentration on both the gelation time and clot quality was examined. Lyophilized bovine fibrinogen (3 mg/ml) was solubilized in various CaCl_2 solutions. No collagen was used in this study. Standard conditions were used (i.e. 37°C test temperature, 1 ml test solution used per $[\text{Ca}^{++}]$ dose level, 1 NIHu thrombin).

TABLE 6
EFFECT OF Ca^{++} ON GELATION TIME,

$[\text{Ca}^{++}]$ (M)	Gel Time (sec)	Comments
0.01	30	fast onset, slow to harden, fragile
0.02	15	firm clot
0.04	10	firm clot
0.06	50	soft clot at 25 sec, then firm
0.08	150	small clots on side of tube, then entire test solution gels
0.10	150	small clots on side of tube, then entire test solution gels
0.20	10 min	
0.40	> 10 min	
0.80	> 10 min	
Ringer's control	21 sec	$[\text{Ca}^{++}] = 0.02 \text{ M}$

The results illustrated in Table 6 show that the calcium ion concentration for optimum gelation time and clot quality ranges between 0.02 M and 0.04 M. This is in agreement with prior published studies.

Example 3

Shelf Life Effects on Gelation

The reagents and gelation conditions used in the study were identical to those described in Example 2. Lyophilized fibrinogen was reconstituted in Ringer's in the following concentrations:

Concentrations of Test Solutions

- fibrinogen: 3 mg/ml in test solution;
- fibrinogen with collagen:
- collagen: 17.5 mg/ml in test solution;
- fibrinogen: 1.5 mg/ml in test solution.

Standard Gelation Conditions Used

- test temperature: 37°C
- amount of test solution: 1 ml
- amount of thrombin: 1 NIHU
- (10 μl of a 100 NIHU solution in sterile water for injection)

TABLE 7
BOVINE FIBRINOGEN—GELATION TIME

	4° C Storage Temp	
	Time Temp Exp (hrs)	-ZCl1 (sec) + ZCl1 (sec)
5	0	20 10
10	24	19 10
	48	18 8
	72	20 10
	96	15 10
	120	15 10
15	168	15 10*

*The fibrinogen appears gelled, even when thawed to 37°C prior to addition of thrombin.

20 Time Temp Exp (hrs) = time of test solution's exposure (or storage) to indicated temperatures (either 22°C or 4°C)

25 -ZCl1 = without ZCl1, [bovine fibrinogen solution without collagen]

+ ZCl1 = with ZCl1 [bovine fibrinogen solution mixed with collagen]

TABLE 8
HUMAN PLASMA—GELATION TIME

	4° C Storage Temp			22° C Storage Temp		
	Time Temp exp (hrs)	-ZCl1 (sec)	+ZCl1 (sec)	Time Temp exp (hrs)	-ZCl1 (sec)	+ZCl1 (sec)
35	0	18	12	0	17	10
40	1	15	*	1	18	11
	3	16	*	2	17	11
	4	15	*	4	14	10
	20	20	*	8	17	12
	30	15	*	24	20	*
45	48	18	*			
	120	17	*			

*Test solution appeared to have gelled prior to addition of thrombin.

50 As demonstrated in the tables, lyophilized fibrinogen with collagen was stable for over 120 hrs at 4°C. Human plasma (normal range of fibrinogen concentration in plasma is about 2 to 4 mg/ml) with collagen gelled spontaneously in less than an hour at 4°C. At room temperature (22°C.), the mixture was stable for up to about 8 hrs.

Example 4

Clot Stability In Vitro

One ml of adhesive material was placed in about 30 ml of Ringer's solution and allowed to sit undisturbed at 37°C until the clot dissolved in its entirety. The adhesive materials compared were:

65 #1 - 0.5 ml human plasma + 0.5 ml ZCl1 collagen + 100 NIH units bovine thrombin in 1 ml of 25 mg/ml

ϵ -aminocaproic acid;

#2 - 0.5 ml human plasma + 0.5 ml ZCII collagen + 100 NIH units bovine thrombin in 1 ml of Sterile Water for Injection, U.S.P.;

#3 - 1.0 ml human plasma + 100 NIH units bovine thrombin in 1 ml of 25 mg/ml ϵ -aminocaproic acid; and

#4 - 1.0 ml human plasma + 100 NIH units bovine thrombin in 1 ml Sterile Water for Injection, U.S.P.

Six replicate clots were formed for each of the four groups. One Ringer's solution control was used for a turbidity assessment.

The data shows that after 35 days, group #1 showed the least degradation, where 2 of 6 clots gave off very small amounts of flocculant material. Group #2 was the second most durable group of clots with 4 of 6 clots which gave off flocculant material. Group #3 was less stable than group #2, with 6 out of 6 clots giving off flocculant material. Group #4 showed the most degradation, with 2 out of 6 clots completely degraded and the remaining clots far more fluid and flocculant than the other groups.

Example 5

Human Clinical Trials

Human clinical trials included 24 patients undergoing a variety of middle ear surgical procedures, as well as one neurologic procedure. The 24 cases were broken down into two phases. Phase I incorporated the use of patient autologous plasma cryoprecipitate with collagen, thrombin and an anti-fibrinolytic agent. Twenty-one subjects were assigned to this first phase. Phase II incorporated the use of patient autologous citrated plasma (without any fibrinogen concentration) with collagen, thrombin and antifibrinolytic. Three subjects have been incorporated into this phase. The surgical procedures and outcome for each test subject in both phases are outlined hereinafter. The preparative steps for each phase is as follows. For Phase I, 10 cc of the patient's blood was collected into two citrated 5 cc Vacutainers. The blood was centrifuged for 10 min at 4000 rpm. Plasma was collected from each tube and transferred to one siliconized Vacutainer using a 20-gauge x 3" spinal needle and a 12 cc syringe. Plasma was stored for 1 hr or more at -20°C , either in a cryofreezer or a circulating acetone bath. Frozen plasma was stored overnight at 4°C to slowly thaw.

On the morning of surgery, thawed plasma with precipitate was centrifuged for 10 min at 4000 rpm. The top about 4.5 ml of the clear plasma fraction was removed with a 20-gauge x 3" spinal needle and 12 cc syringe, leaving the bottom 0.5 ml of plasma with the cryoprecipitate in the Vacutainer tube. Then, 0.5 ml of 10% CaCl_2 solution was added to the cryoprecipitate-containing plasma and mixed thoroughly using a vortex mixer. After mixing, 0.5 ml of the cryoprecipitate/ CaCl_2 solution was removed using a 1 cc tuberculin syringe. That syringe was connected to another 1 cc syringe containing 0.5 ml of ZCII collagen without lidocaine (35 mg/ml) using a syringe-to-syringe connector, and mixed for 5 to 7 passes until the mixture was uniform. The preparation of component 1 was complete. Component 2 was produced as described in Example 1.

For Phase II, about 5 cc of the patient's blood was collected in one citrated 5 cc Vacutainer during the surgical procedure. Usually, the blood was collected by the anesthesiologist from a catheter access port. The collected blood in the citrated Vacutainer was then centrifuged in a clinical centrifuge for 10 minutes at 4000 rpm. 0.5 cc of the plasma fraction was then withdrawn with a 20g X 3+ spinal needle and a 1 cc tuberculin syringe. The spinal needle was removed and a sterile syringe-to-syringe connector was attached. The syringe containing 1 cc of ZCII (without lidocaine) was then attached to the other end of the connector. 0.5 cc of the collagen were then passed to the syringe containing the 0.5 cc of citrated plasma. The collagen-containing syringe was removed from the connector and an empty 1 cc syringe was attached. The plasma/collagen mixture was mixed by passing back and forth for 5 to 10 passes until uniform. Component 2 was produced as described in Example 1. The two component-bearing syringes were then attached to a dual-syringe deliver device, or modified spinal needles were attached to each individual syringe.

TABLE 9
OUTLINE OF SURGERIES, PHASE I

5	Patient Case #	Surgical Procedure	Patient Age (at time of surgery)	Patient Sex	No. of Post-Op Exams (to date)
	1	tympanossiculoplasty	56	F	3
	2	ossiculoplasty revision	62	M	2
10	3	tympanoplasty with mastoidectomy	9	M	3
	4	ossiculoplasty revision with meatoplasty revision	24	F	3
	5	laser stapedotomy	42	M	0
	6	tympanossiculoplasty		M	
15	7	laser stapedotomy		M	
	8	ossiculoplasty	14	F	2
	9	laser stapedotomy	17	M	
	10	external canal exotoses	39	M	3
20	11	laser stapedotomy	58	M	2
	12	laser stapedotomy	43	F	2
	13	laser stapedotomy	47	F	2
	14	ossiculoplasty	10	F	2
	15	tympanomastoid reconstruction	65	F	1
25	16	ossiculoplasty	68	M	1
	17	ossiculoplasty		M	
	18	laser stapedotomy		F	
	19	laser stapedotomy	56	F	2
30	20	laser stapedotomy	49	M	1
	21	tympanossiculoplasty with mastoidectomy		M	

TABLE 10
OUTLINE OF SURGERIES, PHASE II

35	Patient Case #	Surgical Procedure	Patient Age (at time of surgery)	Patient Sex	No. of Post-Op Exams (to date)
	22	laser stapedotomy	44	F	2
	23	ossiculoplasty	29	M	2
40	24	acoustic neuroma	40	F	1

Surgical Procedure Descriptions

External Canal Exotoses:

50 The posterior canal wall skin was elevated and the bony exotoses were removed by use of a pneumatic hand-held de-burring tool. The canal wall skin was then placed back down using the surgical adhesive to adhere it to the bony wall as well as to fill in any gaps in the canal wall skin flaps.

Laser Stapedotomy:

55 The tympanic membrane was dissected partially out of the sulcus, along with some of the adjacent canal wall skin. The crura of the stapes was removed from the footplate by laser dissection. A small hole was then drilled into the center of the stapes footplate by laser. The teflon end of a stapes prosthesis was then introduced into the hole and was glued into place with the surgical adhesive. The platinum wire end of the prosthesis was then wrapped around the incus. Then, the tympanic membrane flap was re-positioned into the sulcus and was adhered in place with the surgical adhesive. No canal packing was used to hold the tympanic membrane in place.

Ossiculoplasty (revision):

65 Generally, an incision was made immediately behind the ear. The tympanic membrane, along with a flap of adjacent canal wall skin was incised and folded up to allow for good exposure and access to the middle ear

space. Depending upon the condition of the ossicles, prosthesis replacing either one, two or all three ossicles were attached to either the tympanic membrane and/or ossicles and glued into position with the surgical adhesive. The tympanic membrane and attached canal wall skin flap were re-positioned and adhered back to their original configuration. The post-auricular incision was then closed with sutures. No canal packing was used to hold the tympanic membrane in place. Revision indicates that this was a repeat of a particular procedure for a patient.

Ossiculoplasty (revision) with Meatoplasty Revision:

The same procedures were followed as described previously, however, prior to replacing the ossicle(s), a number of small incisions were made in the cartilaginous opening of the auditory canal (the meatus) as well as along the canal wall. Pieces of cartilage and small pieces of skin were excised as desired to re-shape and re-contour a deformed meatus and canal. The incisions were re-positioned and adhered with the surgical adhesive. Exposed gaps in the skin closures were filled with the adhesive to close the wounds. The ossiculoplasty then continued as previously described. After the ossiculoplasty procedure, the meatoplasty was again checked to ensure that the desired meatus and canal configuration were maintained and were subsequently packed with antibiotic-soaked dressing.

Tympanoossiculoplasty:

This is another version of the previously described ossiculoplasty. However, the tympanic membrane was replaced with either a homologous tympanic membrane transplant or a piece of patient autologous shaped, dried and sometimes formaldehyde-fixed fascia from the temporalis muscle. The ossicular prosthesis was positioned and glued onto the replacement tympanic membrane. Also, the replacement membrane was glued into position in the sulcus with the surgical adhesive. No canal packing was used.

Tympanoossiculoplasty with Mastoidectomy:

In addition to performing the above-described tympanoossiculoplasty, part of the mastoid bone immediately posterior to the canal wall and middle ear space was rebuilt. Infected mastoid bone was debrided by a pneumatic hand-held drill after exposure of the site by an incision behind the ear. The subsequently formed cavity was then walled off from the middle ear space as well as simultaneously rebuilding the posterior canal wall by a piece of pre-formed, formaldehyde-fixed dura. Osteoblasts were harvested from a donor site in the mastoid bone and were mixed with the surgical adhesive either in situ or during the preparation of the adhesive. This adhesive/osteoblast mixture was then used to hold the pre-formed dura in place. The canal wall skin was then re-positioned and adhered to the dura wall with surgical adhesive after gluing the ossicular prosthesis and homologous tympanic membrane in place.

Tympanomastoid Reconstruction:

This procedure was the same as described previously in the tympanoossiculoplasty with mastoidectomy except that there was no replacement of the ossicles.

Acoustic Neuroma:

An approximately 5 cm diameter hole was drilled into the skull behind the ear. The dura immediately beneath the opening was incised and folded away to reveal the cerebellum and brain stem. The tumorous growth on the acoustic nerve (8th cranial nerve) was located and dissected away with a laser and microdissection instruments. A piece of patient autologous muscle and fascia were coated with the surgical adhesive and were inserted into the internal auditory canal. Additional adhesive was used to obtain a liquid-tight seal to prevent leakage of the cerebro-spinal fluid since the auditory nerve was removed in this procedure.

The use of the present surgical adhesive facilitated quicker healing of TM and canal incisions. Further, it was noted that in an informal comparison of MIPs used with and without the surgical adhesive, a proportionately higher number of slipped prostheses occurred without the use of the adhesive to anchor them in place.

The only disadvantages noted were that there may be a sensitivity reaction of the patient to the collagen component. Current reaction rates to Zyderm I were approximately 20%.

The data demonstrated the following advantages of the present surgical adhesive formulation over various prior autologous fibrinogen glue (AFG) formulations. The surgical adhesive had a higher viscosity than AFG, which allowed more precise placement of the glue and clean-up of excess material. The viscosity was varied depending on the amount of collagen added to provide for a variety of applications. The whitish color of the surgical adhesive stands out in the surgical field unlike AFG which is reddish or straw in color.

A greater adhesiveness and overall durability permitted rougher handling of the adhesive. *In vitro* data showed greater persistence of the clot allowing for better healing. The surgical adhesive was a good tissue filler in the plastic reconstructive aspects of ear surgery. A lower viscosity material (such as found in formulations without the collagen) could not be used to adequately hold a meatoplasty with packing.

The surgical adhesive was much easier and quicker to prepare than current AFG formulations, especially when plasma was used instead of cryoprecipitate as in Phase II of the Clinical Trials. In fact, the surgical adhesive was prepared in the OR as the patient was undergoing anesthesia, unlike current AFG formulations which require overnight processing. The surgical adhesive used far less of the patient's blood than current AFG formulations, which require at least about 40 to 80 cc. In contrast, only 5 cc of plasma were required for

quantities sufficient for middle ear surgery with bone and/or soft tissue reconstruction. A topical antimicrobial agent can be added to the surgical adhesive for percutaneous or external flap closure or augmentation.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

Claims

1. A surgical adhesive useful in treating a patient comprising in an aqueous composition:
 - (a) plasma from said patient;
 - (b) collagen in an amount sufficient to thicken said composition; and
 - (c) thrombin in an amount sufficient to catalyze polymerization of fibrinogen present in said plasma to produce a clot.
2. A surgical adhesive useful in treating a patient comprising in an aqueous composition:
 - (a) plasma from said patient;
 - (b) collagen in a concentration of from about 5 mg/ml to about 30 mg/ml; and
 - (c) from about 1 to about 1000 NIHU thrombin.
3. The surgical adhesive according to claim 1 or claim 2 wherein said collagen concentration is from about 10 mg/ml to about 20 mg/ml.
4. The surgical adhesive according to claim 1, claim 2 or claim 3 additionally comprising an antifibrinolytic agent.
5. The surgical adhesive according to any one of the preceding claims additionally comprising an antimicrobial agent.
6. The surgical adhesive according to any one of the preceding claims additionally comprising an inorganic mineral.
7. The surgical adhesive according to claim 6 wherein said mineral is hydroxyapatite.
8. The surgical adhesive according to claim 6 wherein said inorganic mineral is provided by bone powder or bone chips.
9. The surgical adhesive according to any one of the preceding claims additionally comprising osteoblasts from said patient.
10. A method of making a surgical adhesive for treating a patient comprising:
 - (a) mixing plasma from said patient with collagen in an amount sufficient to thicken said plasma to form a thickened composition;
 - (b) combining said thickened composition with thrombin in an amount sufficient to catalyze polymerization of fibrinogen present in said plasma and optionally an effective amount of an antifibrinolytic agent, whereby said fibrinogen is polymerized to form said surgical adhesive.
11. The method according to claim 10 wherein thrombin is present in a concentration of from about 100 to about 500 NIHU/ml.
12. A kit comprising in a first container flowable, dispersable collagen and in a second container, thrombin, optionally admixed with an antifibrinolytic agent.